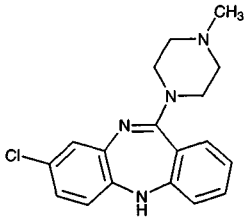
Clozapine



Molecular formula: $C_{18}H_{19}ClN_4$
Molecular weight: 326.83
CAS Registry No.: 5786-21-0
Merck Index: 2484
Lednicer No.: 2 425, 4 212, 220

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum + 4 µg/mL amoxapine in 100 mM HCl + 400 µL 1 M NaOH + 6 mL ethyl acetate, shake for 15 min, centrifuge at 4000 g for 10 min. Transfer top organic layer to another tube and re-extract the analyte with 3 mL 50 mM HCl. Evaporate organic layer under nitrogen at 45°. Dissolve the residue in 120 µL mobile phase, inject a 30 µL aliquot.

HPLC VARIABLES

Guard column: Perisorb RP-8 (Upchurch)
Column: 100 × 4.6 3 µm Supelco C8-DB
Mobile phase: MeCN:MeOH:buffer 20:18:62 (Buffer was 50 mM monobasic sodium phosphate containing 2.5 mL/L triethylamine adjusted to pH 2.7 with phosphoric acid.)
Flow rate: 1.3
Injection volume: 30
Detector: UV 230

CHROMATOGRAM

Retention time: 6.5
Internal standard: amoxapine (14.3)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: amitryptiline, atenolol, carbamazepine, cogentin, desipramine, desmethylsertraline, diazepam, doxepin, fluoxetine, haloperidol, imipramine, loxapine, medazepam, nortryptiline, oxazepam, paroxetine, phenytoin, propranolol, sertraline, thiothixene, trazadone, trifluoperazine, valproic acid, verapamil
Interfering: bupropion

KEY WORDS

serum; pharmacokinetics

REFERENCE

Hariharan,U.; Hariharan,M.; Naickar,J.S.; Tandon,R. Determination of clozapine and its two major metabolites in human serum by liquid chromatography using ultraviolet detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2409–2417.

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. Mix 1 mL plasma or serum with 200 µL 660 nM IS in MeOH:water 5:95. Add to the SPE cartridge. Wash twice with 2 mL water. Acidify the SPE cartridge with 1 mL MeOH:250 mM HCl 10:90, wash with 500 µL MeCN, elute twice with 500 µL 10 mM acetic acid and twice with 500 µL 5 mM diethylamine in MeOH. Evaporate the eluate to dryness with a gentle stream of air at 37°. Reconstitute the residue with 100 µL mobile phase. Inject a 40 µL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm Select-B C8 (Merck)
Mobile phase: MeCN:MeOH:10 mM pH 3.7 dipotassium hydrogen phosphate 30:2:100
Flow rate: 1.5
Injection volume: 40
Detector: UV 220

CHROMATOGRAM
Retention time: 5
Internal standard: protriptyline (8.5)
Limit of detection: 15 nM
Limit of quantitation: 50 nM

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: amitriptyline, clomipramine, chlorpromazine, chlorprothixene, citalopram, desipramine, diazepam, doxepin, fluoxetine, haloperidol, imipramine, levomepromazine, maprotiline, medazepam, mianserin, midazolam, nitrazepam, norclomipramine, nordoxepin, norfluoxetine, normaprotiline, nortrimipramine, nortryptiline, thioridazine, thiothixene, trazodone, trimipramine
Noninterfering: carbamazepine, carbamazepine-10-epoxide, carbamazepine-11-epoxide, clobazam, ethosuximide, flunitrazepam, 10-hydroxycarbazepine, norclobazam, oxazepam, oxcarbazepine, pentobarbital, phenobarbital, primidone, temazepam
Interfering: desmethylcitalopram

KEY WORDS
plasma; serum; SPE; pharmacokinetics

REFERENCE
Åkerman,K.A. Analysis of clozapine and norclozapine by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *696*, 253–259.

SAMPLE
Matrix: blood
Sample preparation: Mix 25 μL 1.5 μg/mL IS, 50 μL serum, and 100 μL 1 M pH 9.0 borate buffer, add 1 mL chloroform, vortex for 1 min, centrifuge at 1100 g for 5 min. Evaporate the organic layer to dryness at 40° under nitrogen, resuspend in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 2.1 5 μm Symmetry C18 Waters
Mobile phase: MeCN:MeOH:buffer 20:10:70 (Buffer was 28.6 mM sodium acetate, pH adjusted to 2.6 with 40% phosphoric acid.)
Flow rate: 0.3
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 4.5
Internal standard: α-hydroxymidazolam (Hoffman-La Roche)(8)
Limit of detection: 2.5 ng/mL
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: N-desmethylclozapine metabolite

KEY WORDS
pharmacokinetics; rat; serum; validation

REFERENCE
Ma,F.; Lau,C.E. Determination of clozapine and its metabolite, N-desmethylclozapine, in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats, *J.Chromatogr.B*, **1998**, *712*, 193–198.

SAMPLE
Matrix: blood
Sample preparation: Mix 400 μL plasma with 50 μL protein releasing reagent. Dialyze 100 μL of this mixture, using a cellulose acetate (Gilson Cuprophane, molecular weight cut-off 15000) membrane, against 4 mL 2 mM pH 4.0 ammonium acetate buffer pumped through a 175 μL channel at 0.47 mL/min. The dialysate flowed through column A to waste. When dialysis is complete elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. Wash the donor channel of the dialysis apparatus with 5 mL 1 mM dodecylethyldimethyl ammonium bromide and the acceptor channel with 5 mL 2 mM pH 4.0 ammonium acetate buffer. (The protein-releasing reagent was aqueous solution of 1 M HCl, 20 mM dodecylethyldimethyl ammonium bromide (Fluka), and 50% (v/v) glycerol.)

HPLC VARIABLES
Column: A 10 × 2 40 μm BondElut C18 (Varian); B 100 × 4.6 5 μm Brownlee CN (Applied Biosystems)
Mobile phase: MeCN:50 mM pH 3.2 ammonium acetate buffer 22:78
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 4.1
Limit of detection: 50 nM
Limit of quantitation: 250 nM

OTHER SUBSTANCES
Extracted: N-desmethylclozapine

KEY WORDS
plasma; dialysis

REFERENCE
Johansen,K.; Krogh,M.; Rasmussen,K.E. Automated on-line dialysis, trace enrichment and high-performance liquid chromatography. Inhibition of interaction with the dialysis membrane and disruption of protein binding in the determination of clozapine in human plasma, *J.Chromatogr.B*, **1997**, *690*, 223–231.

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma or 1 mL diluted red blood cells (red blood cells: water 50:50) with 300 ng loxapine, mix briefly by manual agitation. Add 6 mL ethyl acetate, vortex for 2 min, centrifuge at 2000 g for 5 min. Remove the organic layer, add 250 μL 100 mM HCl, vortex for 2 min, centrifuge at 2000 g for 5 min. Remove a 200 μL volume of the acid layer, evaporate to dryness at 40°. Reconstitute the residue in 200 μL mobile phase and inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Hypersil ODS
Column: 250 × 4.6 5 μm Kromasil Ultrabase C18
Mobile phase: MeCN:buffer 48:52 (Buffer was 716 mg disodium hydrogen phosphate and 2 g cetrimide in 1 L water, adjusted to pH 7.0 with phosphoric acid.)
Column temperature: 50

Flow rate: 1.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 11.0
Internal standard: loxapine (20.0)
Limit of detection: 10 ng/mL
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, amitriptyline, clonazepam, clorazepate, droperidol
Noninterfering: clomipramine, diazepam, hydroxyzine

KEY WORDS
liquid-liquid extraction; plasma; red blood cells; pharmacokinetics

REFERENCE
Guitton,C.; Kinowski,J.-M.; Aznar,R.; Bressolle,F. Determination of clozapine and its major metabolites in human plasma and red blood cells by high-performance liquid chromatography with ultraviolet absorbance detection, *J.Chromatogr.B*, **1997**, *690*, 211–222.

SAMPLE
Matrix: blood
Sample preparation: Add 40 μL 10 μg/mL loxapine to 1 mL plasma, add 200 μL 330 mM NaOH, vortex, add 6 mL hexane:isoamyl alcohol 98.5:1.5, shake for 30 min, centrifuge at 4000 rpm for 5 min. Collect the organic layer, add 150 μL 100 mM HCl, vortex for 1 min. Collect the acidic aqueous phase and inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 C8 (Bischoff, Germany)
Column: 125 × 4.6 5 μm Ecotube Nucleosil C8 (Bischoff, Germany)
Mobile phase: MeCN:Pic B5:water:diethylamine 37:2.5:63:0.04 (Pic B5 is a mixture of water, MeOH, 1-pentanesulfonic acid, and acetic acid and is available from Waters.)
Column temperature: 56
Flow rate: 1.7
Injection volume: 50
Detector: UV 245

CHROMATOGRAM
Retention time: 8.7
Internal standard: loxapine (12.7)
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: alprazolam, bromazepam, chlordiazepoxide, clorazepate, diazepam, flunitrazepam, haloperidol, lorazepam, nitrazepam, oxazepam, paroxetine, prazepam, temazepam, triazolam

KEY WORDS
plasma

REFERENCE
Edno,L.; Combourieu,I.; Cazenave,M.; Tignol,J. Assay for quantitation of clozapine and its metabolite N-desmethylclozapine in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 311–318.

SAMPLE
Matrix: solutions

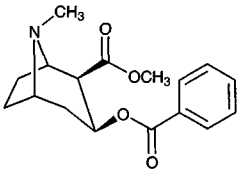
HPLC VARIABLES
Column: 100 × 4.6 Microsorb CN
Mobile phase: MeCN:MeOH:50 mM pH 6.5 sodium phosphate buffer 5:28:67
Column temperature: 37
Flow rate: 1.5

CHROMATOGRAM
Retention time: 12.5

OTHER SUBSTANCES
Simultaneous: olanzapine, paroxetine
Also analyzed: haloperidol, risperidone
Interfering: imipramine

REFERENCE
Prieto,I.V.; Hoffman,D.W. HPLC monitoring of olanzapine (Abstract 131), *Ther.Drug Monit.*, **1997**, *19*, 580–580.

Cocaine



Molecular formula: $C_{17}H_{21}NO_4$
Molecular weight: 303.36
CAS Registry No.: 50-36-2, 53-21-4 (HCl)
Merck Index: 2517

SAMPLE

Matrix: blood
Sample preparation: 100 µL Plasma + 50 µL 5 µg/mL IS + 500 µL pH 6 phosphate buffer + 10 mL chloroform, shake for 10 min (Caution! Chloroform is a carcinogen!). Evaporate the chloroform layer under a stream of nitrogen. Reconstitute the residue in 75 µL mobile phase. Inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 2.1 5 µm Supelcosil ABZ+
Mobile phase: MeCN:50 mM ammonium phosphate 9:91
Flow rate: 0.6
Injection volume: 20
Detector: UV 233

CHROMATOGRAM

Internal standard: lidocaine
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Hedaya,M.A.; Pan,W.-J. Cocaine and alcohol interactions in naive and alcohol-pretreated rats, *Drug Metab.Dispos.*, **1996**, *24*, 807–812.

SAMPLE

Matrix: blood
Sample preparation: Add 1 ml buffer to 1 mL serum, vortex for 5 s. Add 5 ml hexane and shake on an oscillating shaker for 3 min. Centrifuge at 1200 g for 3 min and freeze lower aqueous layer in acetone/dry ice mixture. Evaporate hexane layer to dryness under a stream of nitrogen. Reconstitute with 200 µL mobile phase. Inject an aliquot. (Keep the sample in the autosampler at 2°. Prepare the buffer by mixing 24 mL 100 mM sodium carbonate with 176 mL 100 mM sodium hydrogen carbonate.)

HPLC VARIABLES

Column: 250 × 4.6 5 µm Supelcosil LC-CN
Mobile phase: MeCN:pH 7.4 phosphate buffer 38:62
Flow rate: 1
Detector: UV 230

CHROMATOGRAM

Retention time: 9.3
Internal standard: lidocaine

OTHER SUBSTANCES
Extracted: cocaethylene, cocaine, norcocaine

KEY WORDS
dog; serum; lidocaine is IS

REFERENCE
Williams,C.L.; Laizure,S.C.; Parker,R.B.; Lima,J.J. Quantitation of cocaine and cocaethylene in canine serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *681*, 271–276.

SAMPLE
Matrix: blood
Sample preparation: Mix 50 μL 8 μ g/mL bupivacaine in water with 500 μL pH 6.0 buffer and 100 μL plasma sample, add 10 mL chloroform, shake mechanically for 10 min, centrifuge at 873 g for 10 min, evaporate the organic layer under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 35 μL aliquot. (Prepare pH 6.0 buffer by mixing 67 mM KH_2PO_4 with 67 mM Na_2HPO_4 in an 87.7:12.3 ratio.)

HPLC VARIABLES
Column: 250 × 2.1 5μm Supelcosil ABZ+plus deactivated reversed-phase
Mobile phase: MeOH:MeCN:50 mM pH 4.5 monobasic ammonium phosphate 5:7:63
Flow rate: 0.4
Injection volume: 35
Detector: UV 235

CHROMATOGRAM
Retention time: 12.38
Internal standard: bupivacaine (26.90)
Limit of detection: 24 ng/mL
Limit of quantitation: 81 ng/mL

OTHER SUBSTANCES
Extracted: benzoylecgonine, norcocaine, cocaethylene, metabolites
Simultaneous: ascorbic acid, morphine, oxymorphone, noroxymorphone, norhydromorphone, norcodeine, codeine, nalorphine, procaine, acetaminophen, oxycodone, hydrocodone, caffeine, ethylmorphine, lidocaine, benzoylnorecgonine, ketamine, acepromazine, salicylic acid, benzoic acid, thebaine, cocaine propyl ester, benzocaine, tetracaine, pentobarbital

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Pan,W.-J.; Hedaya,M.A. Sensitive and specific high-performance liquid chromatographic assay with ultraviolet detection for the determination of cocaine and its metabolites in rat plasma, *J.Chromatogr.B*, **1997**, *703*, 129–138.

SAMPLE
Matrix: blood
Sample preparation: Add 50 μL 10 μg/mL lidocaine in MeOH to 1 mL serum, vortex for 5 s. Add 1 ml buffer, vortex for 5 s. Add 5 ml hexane and shake on an oscillating shaker for 3 min. Centrifuge at 1200 g for 3 min and freeze lower aqueous layer in acetone/dry ice mixture. Evaporate hexane layer to dryness under a stream of nitrogen. Reconstitute with 200 μL mobile phase. Inject an aliquot. (Keep the sample in the autosampler at 2° Prepare the buffer by mixing 24 mL 100 mM sodium carbonate with 176 mL 100 mM sodium hydrogen carbonate.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-CN
Mobile phase: MeCN:pH 7.4 phosphate buffer 38:62
Flow rate: 1
Detector: UV 230

CHROMATOGRAM
Retention time: 16.8
Internal standard: lidocaine (9.3)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, cocaethylene, norcocaine

KEY WORDS
dog; serum; pharmacokinetics

REFERENCE
Williams,C.L.; Laizure,S.C.; Parker,R.B.; Lima,J.J. Quantitation of cocaine and cocaethylene in canine serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *681*, 271–276.

SAMPLE
Matrix: blood
Sample preparation: Add 25 μL 4 μg/mL IS to 50 μL serum, add 100 μL 1 M pH 9.0 borate buffer, mix well, add 1 mL EtOH:chloroform 12.5:87.5, vortex for 1 min, centrifuge at 1100 g for 5 min. Evaporate the organic layer to dryness under nitrogen at 40°. Resuspend the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 2.1 5 μm Brownlee C18
Mobile phase: MeCN:MeOH:buffer 10:12.5:77.5 containing 129 μM tetrabutylammonium phosphate (Buffer was 25.8 mM sodium acetate adjusted to pH 2.2 with 40% phosphoric acid.)
Flow rate: 0.3
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: k' 4.37
Internal standard: 3-isobutyl-1-methylxanthine (k' 7.85)
Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, benzoylecgonine, benzoylnorecgonine, norcocaine
Noninterfering: barbital, caffeine, cocaethylene, flurazepam, hexobarbital, lidocaine, mazindol, norcocaethylene

KEY WORDS
pharmacokinetics; rat; serum

REFERENCE
Ma,F.; Zhang,J.; Lau,C.E. Determination of cocaine and its metabolites in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats, *J.Chromatogr.B*, **1997**, *693*, 307–312.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Serum + 200 ng doxepin or desipramine + 100 μL 1 M NaOH + 9 mL freshly prepared hexane:isoamyl alcohol 99:1, shake vigorously for 5 min, centrifuge. Remove 8.5 mL of the organic phase and add it to 200 μL 50 mM HCl, shake well for 1 min, centrifuge, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: MeCN:0.01% phosphoric acid containing 0.01% NaCl 35:65, final pH 2.8
Flow rate: 1.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 5.8
Internal standard: doxepin (12.2), desipramine (14.2)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: dextromoramide, meperidine, methadone, normeperidine, norpropoxyphene, pentazocine, propoxyphene
Simultaneous: amitriptyline, buprenorphine, chlorpromazine, codeine, desmethyldoxepin, diphenhydramine, ephedrine, imipramine, nortriptyline, oxazepam, oxycodone, pericyazine, pheniramine, propranolol, quinine, thiopropazate, thioridazine

KEY WORDS
serum

REFERENCE
Hackett,L.P.; Dusci,L.J.; Ilett,K.F. The analysis of several nonopiate narcotic analgesics and cocaine in serum using high-performance liquid chromatography, *J.Anal.Toxicol.*, **1987**, *11*, 269–271.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 10 μL 25 μg/mL lidocaine in 4 mM HCl, mix, add 100 μL 1 M pH 9.0 borate buffer, add 1 mL chloroform:EtOH 82.5:17.5, mix, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 2 10 μm μBondapak C18
Mobile phase: MeOH:MeCN:buffer 12:16:72 (Buffer was 31 mM sodium acetate adjusted to pH 5.1 with 40% phosphoric acid containing 0.15 mM tetrabutylammonium phosphate.)
Flow rate: 0.3
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 15
Internal standard: lidocaine (10)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: caffeine, metabolites
Simultaneous: barbital, phenobarbital, flumazepil, mazindol, hexobarbital, nicotine, procaine, cotinine
Noninterfering: amphetamine, desipramine, tetracaine, methadone, reserpine, buspirone, diazepam, haloperidol, chlordiazepoxide, oxazepam, midazolam, clonazepam, chlorpromazine, pentobarbital

KEY WORDS
serum; rat

REFERENCE
Lau,C.E.; Ma,F.; Falk,J.L. Simultaneous determination of cocaine and its metabolites with caffeine in rat serum microsamples by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *532*, 95–103.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 40 μg benzoctamine, extract with dichloromethane: isopropanol 80:20

HPLC VARIABLES
Guard column: 30 × 3 7 μm Separon SGX CN (Tessek)
Column: 150 × 3 7 μm Separon SGX CN (Tessek)
Mobile phase: MeOH:buffer 15:85 (Buffer was 100 mM phosphate containing 1 mL/L triethylamine, pH adjusted to 3.5 with phosphoric acid.)
Flow rate: 0.7
Detector: UV 233

CHROMATOGRAM
Retention time: 10
Internal standard: benzoctamine (15)
Limit of detection: 200 ng/mL (urine), 50 ng/mL (serum)

OTHER SUBSTANCES
Extracted: benzoylecgonine

KEY WORDS
serum

REFERENCE
Balíková,M.; Vecerková,J. High-performance liquid chromatographic confirmation of cocaine and benzoylecgonine in biological samples using photodiode-array detection after toxicological screening, *J.Chromatogr.B*, **1994**, *656*, 267–273.

SAMPLE
Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane: isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN:water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: A = MeCN; B = 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 24

Limit of detection: 0.20 ppm

OTHER SUBSTANCES

Extracted: buprenorphine, caffeine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, papaverine, pentazocine, procaine
Also analyzed: bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam, oxazepam

KEY WORDS

whole blood

REFERENCE

Bernal,J.L.; Del Nozal,M.J.; Rosas,V.; Villarino,A. Extraction of basic drugs from whole blood and determination by high performance liquid chromatography, *Chromatographia*, **1994**, *38*, 617–623.

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL Clean Screen SPE cartridge (Worldwide Monitoring) with two 2 mL portions of MeOH, with 3 mL water, and with 3 mL 10 mM pH 3.0 phosphate buffer. 1 mL Serum + bupivacaine + 500 μL 10 mM pH 3.0 phosphate buffer, mix, add to the SPE cartridge, air dry for 30 s, wash with 3 mL phosphate buffer, wash with 3 mL 100 mM HCl, wash with 3 mL MeOH, elute with 2 mL chloroform:isopropanol: ammonium hydroxide 22:20.5:2.5. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.5 SemiPermeable Surface (SPS) C8 (Regis)
Mobile phase: THF:2.5 mM potassium phosphate buffer 3.25:96.75 containing 0.0025% triethylamine, final pH adjusted to 2.7-2.8 with 85% orthophosphoric acid
Flow rate: 0.5
Detector: UV 235

CHROMATOGRAM

Retention time: 15.5
Internal standard: bupivacaine (24)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: acepromazine, atropine, benzoylecgonine, benzoylnorecgonine, ketamine, norcocaine
Noninterfering: benzethonium chloride, benzyl alcohol

KEY WORDS

SPE; serum

REFERENCE

Muztar,J.; Chari,G.; Bhat,R.; Ramaro,S.; Vidyasagar,D. A high-performance liquid chromatographic procedure for the separation of cocaine and some of its metabolites from acepromazine, ketamine, and atropine from serum, *J.Liq.Chromatogr.*, **1995**, *18*, 2635–2645.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min,

inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 231

CHROMATOGRAM

Retention time: 4.75
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: Condition a 300 mg Bond Elut Certify SPE cartridge with 6 mL MeOH, 3 mL water, and 5 mL 10 mM pH 2.0 NaH_2PO_4. 1 mL Plasma + 100 μL 20 μg/mL tropacocaine in water + 3 mL 10 mM pH 2.0 NaH_2PO_4, vortex briefly, add to the SPE cartridge at 0.5 mL/min, dry under vacuum for 1-2 min, wash with 3 mL water at 1 mL/min, dry under vacuum for 3 min, wash with 3 mL 100 mM HCl, dry under vacuum for 3 min, wash rapidly with 6 mL MeOH, dry under vacuum for 5 min, elute with 6 mL dichloromethane:isopropanol:ammonium hydroxide 80:20:2 without vacuum. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 2 mL dichloromethane:isopropanol 80:20. Remove a 1 mL aliquot and evaporate it to dryness under a stream of nitrogen, reconstitute with 300 μL mobile phase, mix for 30 s, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Brownlee C8

Column: 250 × 4.6 5 μm Nucleosil C18

Mobile phase: MeCN:buffer:triethylamine 18:81.7:0.3 (Buffer was 50 mM citric acid:100 mM Na_2HPO_4 80:20, pH 3.0.)

Flow rate: 1.5

Injection volume: 100

Detector: UV 235

CHROMATOGRAM

Retention time: 17.6

Internal standard: tropacocaine (12.8)

Limit of detection: 75 ng/mL

Limit of quantitation: 220 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, benzoylecgonine, norcocaine

KEY WORDS

rat; plasma; SPE

REFERENCE

Virag,L.; Mets,B.; Jamdar,S. Determination of cocaine, norcocaine, benzoylecgonine and ecgonine methyl ester in rat plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1996**, *681*, 263–269.

SAMPLE

Matrix: blood, CSF, gastric contents, urine

Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.

Column temperature: 50

Flow rate: 1.5

Detector: UV 220

CHROMATOGRAM
Retention time: 8.97
Internal standard: heptanophenone (19)

OTHER SUBSTANCES
Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS
serum; column-switching

REFERENCE
Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE
Matrix: blood, hair
Sample preparation: Hair. Wash 20-200 mg hair twice with 20 mL aliquots of 0.3% Tween 20 in water, rinse thoroughly with water, dry at 37°, add 2 mL 250 mM HCl, heat at 45° overnight, neutralize with 1 M NaOH, extract twice with Isolute Confirm HCX (from IST, U.K.), combine the organic layers and evaporate them to dryness, reconstitute with 500 μL 50 mM pH 5.2 NaH_2PO_4, inject a 100-200 μL aliquot. Plasma. 200 μL Plasma + 150 μL 100 mM pH 8.9 Na_2HPO_4 + 5 mL chloroform:isopropanol 90:10, vortex for 2 min, centrifuge at 700 g for 10 min. Evaporate 4 mL of the organic phase, reconstitute the residue in 500 μL pH 5.2 50 mM NaH_2PO_4, inject a 100-200 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Bio-Gel PRP 70-5 poly(styrene-divinylbenzene) (Bio-Rad)
Mobile phase: MeOH:THF:100 mM pH 3 potassium phosphate 25:5:70
Flow rate: 0.5
Injection volume: 100-200
Detector: F ex 230 em 315

CHROMATOGRAM
Retention time: 20
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, amitriptyline, amobarbital, amphetamine, aprobarbital, atropine, barbital, benzoylecgonine, benztropine, butabarbital, caffeine, carbamazepine, carisoprodol, chlorpheniramine, chlorpromazine, chlorprothixene, cimetidine, codeine, dextromethorphan, diazepam, dihydrocodeine, diphenhydramine, diphenoxilate, disopyramide, doxepin, doxylamine, emetine, erythromycin, ethinamate, ethylmorphine, flurazepam, glutethimide, hydrocodone, hydrocortisone, hydromorphone, hydroxyzine, imipramine, lidocaine, loxapine, meperidine, meprobamate, methadone, methamphetamine, methapyrilene, methaqualone, methocarbamol, methylphenidate, morphine, naloxone, nicotine, nordiazepam, nortriptyline, orphenadrine, oxycodone, papaverine, pentazocine, pentobarbital, phenacetin, phencyclidine, phenmetrazine, phenobarbital, phenolphthalein, phentermine, phenylpropanolamine, phenytoin, phetidine, prazepam, procainamide, procaine, propoxyphene, propranolol, protriptyline, pseudoephedrine, pyrilamine, quinine,

salicylamide, secobarbital, spironolactone, strychnine, terpin hydrate, thioridazine, thiothixene, triamterene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine, trimethoprim, trimetobenzamide

KEY WORDS
plasma

REFERENCE
Tagliaro,F.; Antonioli,C.; De Battisti,Z.; Ghielmi,S.; Marigo,M. Reversed-phase high-performance liquid chromatographic determination of cocaine in plasma and human hair with direct fluorimetric detection, *J.Chromatogr.A*, **1994**, *674*, 207–215.

SAMPLE
Matrix: blood, saliva, tissue, urine

Sample preparation: Homogenize (Polytron) tissue with 4 (whole brain) or 8 (brain striata) volumes of 100 mM pH 4.5 NaH_2PO_4 containing 0.5% NaF. Add 500 μL brain homogenate or 500 μL plasma, saliva, or urine containing 15 μL saturated NaF solution to 75 μL 150 μg/mL IS, add 50 μL 50% perchloric acid, mix vigorously for 10 s, let stand at room temperature for 10 min, add 1 mL water, mix briefly, centrifuge at 10° at 2500 (?) for 30 min. Remove the supernatant and add it to 750 μL saturated sodium carbonate solution, mix briefly, add 7.5 mL pentane:chloroform 95:5, rock gently for 10 min, centrifuge in a desk-top centrifuge for 2 min, freeze in dry ice/acetone for 2 min. Remove the organic layer and add it to 250 μL 100 mM HCl, mix vigorously for 10 s, centrifuge in a desk-top centrifuge for 1-2 min, freeze in dry ice/acetone for 3-5 min, discard the organic layer. Allow the aqueous layer to thaw, remove any trace of organic solvent with a stream of nitrogen, inject a 75 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Brownlee RP-8

Column: 250 × 4.6 5 μm Zorbax RX-C18

Mobile phase: MeCN:buffer 18:82 (Buffer was 100 mM K_2HPO_4 containing 0.5% triethylamine, adjusted to pH 2.7 with phosphoric acid.)

Flow rate: 2

Injection volume: 75

Detector: UV 235

CHROMATOGRAM
Retention time: 6.5

Internal standard: 2β-carbomethoxy-3β-(4-chlorophenyl)tropane (RTI-31) (Research Biochemical International, Natick MA) (11.4)

Limit of detection: 5 ng/g (brain), 5 ng/mL (plasma)

Limit of quantitation: 25 ng/g (brain), 25 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: chlordiazepoxide, clozapine, gepirone, methylphenidate, pentazocine, pseudococaine

Simultaneous: acetaminophen, acetophenazine, amoxapine, amphetamine, atropine, benperidol, buspirone, caffeine, carbamazepine, chlorpheniramine, codeine, dextromethorphan, diazepam, diphenhydramine, flupenthixol, flurazepam, haloperidol, hydergine, hydrocodone, hydromorphone, lidocaine, loxapine, mepazine, meperidine, mesoridazine, methaquaalude, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxyethylamphetamine, 3,4-methylenedioxymethamphetamine, morphine, norcocaine, oxazepam, pentobarbital, phenylpropanolamine, procainamide, procaine, propyl benzoylecgonine, quinidine, quinine, salicylic acid, secobarbital, theophylline, trazodone, 3-tropanyl-3,5-dichlorobenzoate, vancomycin, WIN 35428

Noninterfering: amitriptyline, benztropine methanesulfonate, butaperazine, butriptyline, carphenazine, chlorpromazine, clomipramine, cyclobenzaprine, dextropropoxyphene, dronabinol, ephedrine, ethchlorvynol, fluoxetine, fluphenazine, imipramine, meprobam-

ate, methadone, methamphetamine, nicotine, norfluoxetine, nortriptyline, PCP, phenothiazine, pseudoephedrine

KEY WORDS
rat; cow; plasma; brain

REFERENCE
Bonate,P.L.; Davis,C.M.; Silverman,P.B.; Swann,A. Determination of cocaine in biological matrices using reversed phase HPLC: Application to plasma and brain tissue, *J.Liq.Chromatogr.*, **1995**, *18*, 3473–3494.

SAMPLE
Matrix: blood, urine
Sample preparation: Urine. Condition a Bond-Elut Certify SPE cartridge with 2 mL MeOH and 2 mL pH 6 phosphate buffer. Mix 2.5 mL urine and 2.5 mL water, add to the SPE cartridge, wash with 3 mL water, wash with 3 mL 100 mM HCl, wash with 9 mL MeOH, wash with 3 mL ammonia, dry column under vacuum for 5 min, elute with 2 mL chloroform:isopropanol 80:20. Evaporate 1.5 mL of the eluate to dryness under a stream of nitrogen at 65°, reconstitute with 500 μL 1 μg/mL methaqualone in mobile phase, inject a 25 μL aliquot. Plasma. Condition a Bond-Elut Certify SPE cartridge with 2 mL MeOH and 2 mL pH 6 phosphate buffer. Mix 1 mL plasma and 2 mL water, add to the SPE cartridge, wash with 3 mL water, wash with 3 mL 100 mM HCl, wash with 9 mL MeOH, wash with 3 mL ammonia, dry column under vacuum for 5 min, elute with 2 mL chloroform:isopropanol 80:20. Evaporate 1.5 mL of the eluate to dryness under a stream of nitrogen at 65°, reconstitute with 100 μL 1 μg/mL methaqualone in mobile phase, inject a 25 μL aliquot. (Prepare pH 6 phosphate buffer by adding 55 mL 100 mM Na_2HPO_4 to 445 mL 100 mM KH_2PO_4.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher RP18
Column: 125 × 4 5 μm LiChrospher RP-18
Mobile phase: MeOH:pH 7 phosphate buffer 70:30 (Prepare buffer by adding 320 mL 20 mM K_2HPO_4 to 680 mL 20 mM KH_2PO_4.)
Flow rate: From 0.4 to 0.7 over 6 min, to 1.0 over 2 min, to 0.7 over 2 min, to 0.6 over 1 min, to 0.4 over 1 min
Injection volume: 25
Detector: UV 235

CHROMATOGRAM
Retention time: 8.8
Internal standard: methaqualone (5.8)
Limit of detection: 5 ng/mL (urine), 12.5 ng/mL (plasma)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: benzoylecgonine
Simultaneous: acetaminophen, amitriptyline, atropine, benzocaine, caffeine, codeine, diazepam, flunitrazepam, imipramine, ketazolam, lidocaine, meperidine, methadone, midazolam, morphine, nordiazepam, oxazepam, pentobarbital, perphenazine, phenobarbital, procaine, propoxyphene, secobarbital, tetracaine, tetrazepam
Noninterfering: amphetamine, clomipramine, haloperidol, maprotiline, mianserin, thioridazine

KEY WORDS
SPE

REFERENCE
Fernandez,P.; Lafuente,N.; Bermejo,A.M.; Lopez-Rivadulla,M.; Cruz,A. HPLC determination of cocaine and benzoylecgonine in plasma and urine from drug abusers, *J.Anal.Toxicol.*, **1996**, *20*, 224–228.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Whole blood, plasma, or urine + 10 μL 100 μg/mL bupivacaine + 1 mL buffer + 8 mL chloroform:isopropanol 60:40, shake for 10 min, centrifuge. Remove the organic layer and add it to 2 mL 100 mM sulfuric acid, shake for 10 min, centrifuge. Remove the aqueous layer and add it to 2 mL buffer and 10 mL chloroform:isopropanol 60:40, shake for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 40 μL aliquot. (Buffer was saturated sodium carbonate:saturated sodium bicarbonate 50:50.)

HPLC VARIABLES
Guard column: 5 × 6 μBondapak Guard Pak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:100 mM ammonium acetate 30:30:40
Flow rate: 1
Injection volume: 40
Detector: UV 230

CHROMATOGRAM
Retention time: 8
Internal standard: bupivacaine (11)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: benzoylecgonine
Interfering: lidocaine

KEY WORDS
whole blood; plasma

REFERENCE
Rop,P.P.; Grimaldi,F.; Bresson,M.; Fornaris,M.; Viala,A. Liquid chromatographic analysis of cocaine, benzoylecgonine, local anaesthetic agents and some of their metabolites in biological fluids, *J.Liq.Chromatogr.*, **1993**, *16*, 2797–2811.

SAMPLE
Matrix: blood, urine
Sample preparation: 100 μL Plasma or 500 μL urine + 50 μL 1 μg/mL tropacocaine in water + 200 μL 500 mM pH 9.1 carbonate buffer + 2 mL ethyl acetate, shake for 10 min, centrifuge at 1200 g for 3 min. Remove the organic phase and add it to 150 μL 50 mM HCl, shake for 10 min, centrifuge at 1200 g for 3 min, evaporate the aqueous phase with a centrifugal evaporator, reconstitute the residue with 150 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C8
Mobile phase: MeCN:buffer 24:76 (Buffer was 50 mM pH 6.0 KH_2PO_4 containing 100 mM pentanesulfonic acid.)
Flow rate: 1.3
Injection volume: 100
Detector: UV 235

CHROMATOGRAM
Retention time: 8.0
Internal standard: tropacocaine (6.3)
Limit of quantitation: 5 ng/mL (urine), 25 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites, cocaethylene, norcocaethylene, norcocaine

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Sukbuntherng,J.; Walters,A.; Chow,H.-H.; Mayersohn,M. Quantitative determination of cocaine, cocaethylene (ethylcocaine), and metabolites in plasma and urine by high-performance liquid chromatography, *J.Pharm.Sci.*, **1995**, *84*, 799–804.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Condition a Bond Elut C8 SPE cartridge with 3 mL MeOH, two 3 mL portions of water, and 2 mL buffer. 100 μL Plasma or serum + 100 μL MeOH + 200 μL MeCN + 100 μL buffer, vortex for 1 min, centrifuge at 4000 rpm for 15 min, add the supernatant to the SPE cartridge, wash with two 3 mL portions of water, dry under vacuum for 10 min, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 5 μg/mL nalorphine in MeOH (?), inject an aliquot. Urine. Condition a Bond Elut C8 SPE cartridge with 3 mL MeOH, two 3 mL portions of water, and 2 mL buffer. 100 μL Urine + 100 μL MeOH + 200 μL MeCN + 500 μL buffer, vortex for 1 min, centrifuge at 2000 rpm for 5 min, add the supernatant to the SPE cartridge, wash with two 3 mL portions of water, dry under vacuum for 10 min, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 5 μg/mL nalorphine in MeOH (?), inject an aliquot. (Buffer was 250 mL 25 mM sodium borate and 18 mL 100 mM NaOH, pH 9.2.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Adsorbosphere HS C18
Mobile phase: MeCN:MeOH:1.2% ammonium acetate 15:40:45
Flow rate: 0.8
Detector: UV 239

CHROMATOGRAM
Retention time: 14.55
Internal standard: nalorphine (9.72)
Limit of quantitation: 83 ng/mL (urine), 100 ng/mL (plasma, serum)

OTHER SUBSTANCES
Extracted: benzoylecgonine

KEY WORDS
SPE; plasma; serum

REFERENCE
Theodoridis,G.; Papadoyannis,I.; Tsoukali-Papadopoulou,H.; Vasilikiotis,G. A comparative study of different solid phase extraction procedures for the analysis of alkaloids of forensic interest in biological fluids by RP-HPLC/Diode array, *J.Liq.Chromatogr.*, **1995**, *18*, 1973–1975.

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 11.92

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Inject a 200 μL aliquot.

HPLC VARIABLES
Column: 100 × 5 10 μm Radial-Pak C18 radial compression (Waters)
Mobile phase: MeOH:100 mM NaCl:butylamine 50:50:0.7, adjusted to pH 3 with sulfuric acid
Flow rate: 1
Injection volume: 200
Detector: E, Bioanalytical systems LC4B, MF 1000 dual glassy carbon working electrode + 1.1 V and +0.75 V, MF 1018 stainless steel auxiliary electrode, MF 2020 Ag/AgCl reference electrode following post-column reaction. The column flowed through a knitted 9.1 m × 0.5 mm ID PTFE coil irradiated at 254 nm by a Photronix medium-pressure mercury lamp (cooled with ice-water) to the detector.

CHROMATOGRAM
Retention time: k' 0.53
Limit of detection: 500 ppb

OTHER SUBSTANCES
Simultaneous: amylocaine, ascorbic acid, caffeine, chloroprocaine, lidocaine, niacinamide, procaine
Noninterfering: inositol, lactose, mannitol

KEY WORDS
post-column reaction; post-column photochemical derivatization

REFERENCE
Selavka,C.M.; Krull,I.S.; Lurie,I.S. An improved method for the rapid screening of illicit cocaine preparations using high performance liquid chromatography with electrochemical detection, *Forensic Sci.Int.*, **1986**, *31*, 103–117.

SAMPLE
Matrix: bulk
Sample preparation: Weigh out 50 mg of the hydrochloride, dissolve in 2 mL 24 μg/mL meconin and 60 μg/mL n-butyrophenone in MeCN:buffer 20:80, vortex, inject a 150 μL aliquot. (Buffer was 40 mL phosphoric acid and 120 mL 2 mM NaOH in 3480 mL water containing 20 mM sodium dodecyl sulfate, pH 2.0.)

HPLC VARIABLES
Column: 125 × 4.6 HS-5 C18 (Perkin-Elmer)
Mobile phase: Gradient. MeCN:THF:buffer 21.9:1.7:76.4 to MeCN:buffer 44:56 over 12 min (concave gradient), to MeOH:buffer 80:20 over 13 min (linear), maintain at MeOH:buffer 80:20 for 8 min, return to initial conditions over 5 min. (Buffer was 40 mL phosphoric acid and 120 mL 2 mM NaOH in 3480 mL water containing 20 mM sodium dodecyl sulfate, pH 2.0.)
Flow rate: 1.5
Injection volume: 150
Detector: UV 215 and 277

CHROMATOGRAM
Retention time: 15
Internal standard: meconin (2.5), n-butyrophenone (11.5)

OTHER SUBSTANCES
Simultaneous: impurities, benzoylecgonine

REFERENCE
Lurie,I.S.; Moore,J.M.; Cooper,D.A.; Kram,T.C. Analysis of manufacturing by-products and impurities in illicit cocaine via high-performance liquid chromatography and photodiode array detection, *J.Chromatogr.*, **1987**, *405*, 273–281.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 750 μg/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100
Column: 125 × 4 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was water containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. B was MeCN:water 90:10 containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. A:B from 91:9 to 86:14 over 4 min, maintain at 86:14 for 13 min, to 55:45 over 11 min, maintain at 55:45 for 8 min, re-equilibrate at initial conditions for 20 min.
Flow rate: 0.7
Injection volume: 15
Detector: UV 210

CHROMATOGRAM
Retention time: 26.3

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetylcodeine, benzocaine, caffeine, codeine, diamorphine, lidocaine, 6-monoacetylmorphine, morphine, noscapine, papaverine, procaine

REFERENCE
Grogg-Sulser,K.; Helmlin,H.-J.; Clerc,J.-T. Qualitative and quantitative determination of illicit heroin street samples by reversed-phase high-performance liquid chromatography: method development by CARTAGO-S, *J.Chromatogr.A*, **1995**, *692*, 121–129.

SAMPLE
Matrix: dialysate
Sample preparation: Inject a 5 μL aliquot of dialysate.

HPLC VARIABLES
Column: 100 × 1 3 μm SepStik (BioAnalytical Systems, West Lafayette, IN)
Mobile phase: THF:50 mM NaH_2PO_4 24:76 containing 0.2 mM disodium EDTA, 3.7 mM 1-decanesulfonic acid, and 4.9 mM triethylamine, pH 5.5
Flow rate: 0.023
Injection volume: 5
Detector: E, (EG & G Princeton Applied, USA), Model MP 1304, glassy carbon electrode, first at +700 mV, second at 0 mV, Ag/AgCl reference electrode (BioAnalytical Systems, Model RE4); UV 225

CHROMATOGRAM
Retention time: 25
Limit of quantitation: 100 nM

OTHER SUBSTANCES
Simultaneous: dopamine

KEY WORDS
rat; brain; microdialysis; pharmacokinetics

REFERENCE
Parsons,L.H.; Kerr,T.M.; Weiss,F. Simple microbore high-performance liquid chromatographic method for the determination of dopamine and cocaine from a single in vivo brain microdialysis sample, *J.Chromatogr.B*, **1998**, *709*, 35–45.

SAMPLE
Matrix: leaves
Sample preparation: Reflux 0.05-4 g air-dried coca leaves in 30-100 mL 95% EtOH for 15 min, filter (Whatman No. 4 paper), inject an aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm C8 (R.E.Gourley)
Mobile phase: MeCN:1% triethylamine pH 4 40:60
Flow rate: 1.2
Injection volume: 5
Detector: UV 240

CHROMATOGRAM
Retention time: 2.5
Limit of detection: 5 ng

REFERENCE
Glass,R.L.; Johnson,E.L. Comparison of high performance liquid chromatographic and gas chromatographic analyses of cocaine in coca leaves, *J.Liq.Chromatogr.*, **1993**, *16*, 3543–3555.

SAMPLE
Matrix: meconium
Sample preparation: Condition a 1 mL 100 mg Bond Elut strong cation exchange SPE cartridge with 2 mL MeOH, 1 mL water, and 1 mL 250 mM phosphate buffer. 0.5 g Meconium + 2 mL MeOH, vortex for 1 min, centrifuge at 450 g for 5 min. Remove the supernatant and add it to 1 mL 25 mM pH 3 potassium phosphate buffer, add to SPE cartridge, air dry for 30 s, wash with 1 mL phosphate buffer, wash with 100 mM acetic acid, air dry for 30 s, elute with 2 mL 3% ammonia in MeOH, evaporate eluate to dryness under a stream of nitrogen, reconstitute in 50 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 4.5 C18 Guard Pak (Waters)
Column: 250 × 4.5 5 μm Spherisorb ODS
Mobile phase: MeCN:25 mM potassium phosphate buffer 15:85 containing 25 mL/L diethylamine, final pH adjusted to 2.9 with concentrated orthophosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 230, 255, 270

CHROMATOGRAM
Retention time: 11.0

OTHER SUBSTANCES
Extracted: benzoylecgonine

KEY WORDS
SPE

REFERENCE
Browne,S.P.; Tebbett,I.R.; Moore,C.M.; Dusick,A.; Covert,R.; Yee,G.T. Analysis of meconium for cocaine in neonates, *J.Chromatogr.*, **1992**, *575*, 158–161.

SAMPLE
Matrix: meconium
Sample preparation: Condition a 200 mg Clean-Screen DAU SPE cartridge (Worldwide Monitoring) with two 3 mL portions of MeOH, 3 mL water, and 3 mL pH 3 buffer. 0.5 g Meconium + 2 mL MeOH, vortex, centrifuge. Remove the supernatant and add it to 3 mL pH 3 phosphate buffer and 20 μL 100 ng/mL bupivacaine, mix, add to the SPE cartridge, air dry, wash with 3 mL buffer, wash with 3 mL 100 mM HCl, wash with 3 mL MeOH, elute with three 1 mL portions of chloroform:isopropanol:ammonium hydroxide 78:20:2. Evaporate the eluate to dryness without heating, reconstitute the residue in 100 μL MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: Guard-Pak C18 (Waters)
Column: 300 × 3.9 μBondapak C18 ODS
Mobile phase: MeCN:butylamine:25 mM KH_2PO_4 125:12.5:500, pH adjusted to 2.9 with 85% phosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 230, UV 255, UV 275

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE

REFERENCE

Browne,S.; Moore,C.; Negrusz,A.; Tebbett,I.; Covert,R.; Dusick,A. Detection of cocaine, norcocaine, and cocaethylene in the meconium of premature neonates, *J.Forensic Sci.*, **1994**, *39*, 1515–1519.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.2

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimetho-

benzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH or water to 0.1%.

HPLC VARIABLES

Column: two 250 mm β-cyclodextrin bonded phase columns in series (Advanced Separation Technologies)
Mobile phase: MeCN:1% pH 4.1 aqueous triethylammonium acetate 4:96
Flow rate: 0.5
Injection volume: 1
Detector: UV

CHROMATOGRAM

Retention time: 87 (d-isomer), 91 (l-isomer)

OTHER SUBSTANCES

Simultaneous: scopolamine
Interfering: atropine

KEY WORDS

chiral

REFERENCE

Armstrong,D.W.; Han,S.M.; Han,Y.I. Separation of optical isomers of scopolamine, cocaine, homatropine, and atropine, *Anal.Biochem.*, **1987**, *167*, 261–264.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 10 μm PRP-1 (Hamilton)
Mobile phase: Gradient. MeCN:20 mM ammonium hydroxide from 15:85 to 100:0 over 17 min
Flow rate: 1
Detector: UV 220

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Simultaneous: codeine, methadone, reserpine, thebaine, yohimbine

REFERENCE

Keystone Scientific Catalog, 1993-4, p. 22.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM

Retention time: 3.905

OTHER SUBSTANCES

Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE

Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993,** *12(3)*, 18–21.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, me-

phobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 10.00 (A), 4.99 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac,

labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: Condition a Varian 1 mL SCX SPE ion exchange cartridge with 2 mL MeOH, 1 mL water, and 1 mL 250 mM pH 3.0 phosphate buffer. Dilute urine specimens 1:1 with 250 mM phosphate buffer, add 500 ng/mL IS, add to SPE cartridge, dry under vacuum for 30 s, wash with 1 mL phosphate buffer, wash with 500 μL 100 mM acetic acid, wash with 1 mL MeOH. Dry column again for 30 s, elute with 1.5 mL 3% ammonium hydroxide in MeOH. Evaporate the eluate to dryness under nitrogen, reconstitute with 100 μL MeOH, evaporate half of this solution to dryness under a stream of nitrogen at 60-70°, reconstitute in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: Novapak Guard pak precolumn
Column: 250 × 4.6 10 μm Lichrosorb RP-18
Mobile phase: MeCN:25 mM KH_2PO_4 buffer: butylamine (18:81:1, v/v/v), adjusted to pH 3.0 with orthophosphoric acid
Flow rate: 1.5
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 10.27
Internal standard: bupivacaine (15.55)
Limit of detection: 1 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, benzoylecgonine, cocaethylene, norcocaine

KEY WORDS
SPE; comparison with GC-MS

REFERENCE

Phillips,D.L.; Tebbett,I.R.; Bertholf,R.L. Comparison of HPLC and GC-MS for measurement of cocaine and metabolites in human urine, *J.Anal.Toxicol.*, **1996**, *20*, 305–308.

SAMPLE

Matrix: urine

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH, 3 mL MeCN:10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, and 5 mL water. 5 mL Urine + 5 mL 500 mM ammonium acetate, adjusted to pH 9.5 with ammonia, mix, add to the SPE cartridge, wash with 20 mL 5 mM pH 9.5 ammonium acetate, wash with 0.5 mL water. Elute with 2 mL MeCN:10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES

Column: 150 × 4.6 L-column ODS (Chemical Inspection & Testing Institute, Tokyo)

Mobile phase: Gradient. MeCN:100 mM ammonium acetate 0:100 for 1 min, to 40:60 over 20 min.

Flow rate: 1

Injection volume: 50

Detector: UV 210; MS Shimadzu model QP-1100EX thermospray, vaporizer temperature from 170 to 150° over 20 min. SIM, m/z 304

CHROMATOGRAM

Retention time: 21

Limit of detection: 2–40 ng/mL

OTHER SUBSTANCES

Extracted: 6-acetylmorphine, amphetamine, benzoylecgonine, ephedrine methamphetamine, methylephedrine, morphine, morphine-3-glucuronide, morphine-6-glucuronide

KEY WORDS

SPE

REFERENCE

Tatsuno,M.; Nishikawa,M.; Katagi,M.; Tsuchihashi,H. Simultaneous determination of illicit drugs in human urine by liquid chromatography-mass spectrometry, *J.Anal.Toxicol.*, **1996**, *20*, 281–286.

SAMPLE

Matrix: urine

Sample preparation: Condition a 3 mL Bond Elut Certify C8/SCX SPE cartridge with 3 mL MeOH and 3 mL 100 mM pH 6 phosphate buffer (all flow rates are 1 mL/min). Mix 2 mL urine, 3 mL water, 2 mL 100 mM pH 6 phosphate buffer, and 100 μL internal standard solution (containing 13 μg/mL of both 2′-methylbenzoylecgonine and 2′-methylcocaine), adjust pH to 4.0-6.0 if necessary, add to the SPE cartridge, wash with 3 mL HPLC water, 3 mL 100 mM HCl, three 3 mL portions of MeOH and three 3 mL portions of MeCN. Elute with 2 mL dichloromethane:2-propanol:25% ammonium hydroxide 80:20:2. Evaporate the eluate at 35° under a stream of nitrogen.

HPLC VARIABLES

Guard column: 7.5 × 4.6 5 μm Hypersil BDS C18

Column: 150 × 4.6 5 μm Hypersil BDS C18

Mobile phase: Gradient. A was MeCN:MeOH:water 10:10:80 containing 45 mM ammonium acetate. B was MeCN:MeOH:water 40:40:20. A:B from 100:0 to 47.2:52.8 in 19 min, maintain at 47.2:57.8 for 2 min, to 100:0 in 1 min, maintain at 100:0 for 5 min.

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM
Retention time: 13.7
Internal standard: 2′-methylbenzoylecgonine (9.7), 2′-methylcocaine (17.7) [Synthesis described in paper.]
Limit of detection: 20 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: acetaminophen, acetylcodeine, alprenolol, amphetamine, apomorphine, bromazepam, caffeine, citalopram, codeine, dihydrocodeine, hydrocodone, hydromorphone, methamphetamine, methylephedrine, monoacetylmorphine, morphine, naloxone, nitrazepam, oxycodone, phenobarbital, pholcodine, pindolol, propranolol, viloxazine
Noninterfering: alprazolam, amitriptyline, brotizolam, butryptiline, camazepam, chlordiazepoxide, clobazam, clomipramine, clonazepam, clotiazepam, cloxazolam, desipramine, diazepam, dothiepin, doxepin, ethyl loflazepate, flunitrazepam, fluoxetine, flurazepam, fluvoxamine, halazepam, imipramine, lofepramine, loprazolam, lorazepam, lormetazepam, maprotiline, medazepam, melitracen, mianserin, midazolam, nordazepam, nortriptyline, opipramol, oxazepam, paroxetine, sertraline, temazepam, trazodone, triazolam, trimipramine
Interfering: thebacon

KEY WORDS
SPE

REFERENCE
Clauwaert,K.M.; Van Bocxlaer,J.F.; Lambert,W.E.; De Leenheer,A.P. Analysis of cocaine, benzoylecgonine, and cocaethylene in urine by HPLC with diode array detection, *Anal.Chem.*, **1996**, *68*, 3021–3028.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 3 mL 5 M NaOH, vortex 30 s, add 12 mL diethyl ether, rotate for 5 min, centrifuge at 2500 rpm for 5 min. Remove the ether layer and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute in 2 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Alltech C18
Mobile phase: MeOH:water 50:50 containing 7 mL/L butylamine, adjusted to pH 3.2 with sulfuric acid
Flow rate: 1.8
Injection volume: 50
Detector: E, Bioanalytical Systems Model LC4B, dual glassy carbon working electrode cell half operated in the parallel mode + 1.0 V and +0.9 V, stainless steel auxiliary electrode cell half, Ag/AgCl reference electrode. The detector was preceded by a Photronix Model 816 UV irradiator which irradiated the mobile phase in a 9.144 m length of 0.5 mm i.d. × 1.6 mm o.d. Teflon tubing in a three-dimensional figure eight configuration. The irradiation apparatus was maintained at 0-5° using an ice bath.

CHROMATOGRAM
Retention time: 6
Limit of detection: 50 ppb

OTHER SUBSTANCES
Simultaneous: methylphenidate, phenobarbital, nitrazepam
Interfering: chlordiazepoxide

REFERENCE

Selavka,C.M.; Krull,I.S.; Lurie,I.S. Photolytic derivatization for improved LCEC determinations of pharmaceuticals in biological fluids, *J.Chromatogr.Sci.*, **1985**, *23*, 499–508.

SAMPLE

Matrix: urine

Sample preparation: 1 mL Urine + 0.5 mL 1% trichloroacetic acid, centrifuge at 5200 g for 10 min, filter (0.2 μm), inject 20 μL aliquot

HPLC VARIABLES

Column: 250 × 4 Lichrospher 5μm 60 RP-select B

Mobile phase: Gradient. MeCN:50 mM pH 3.2 potassium phosphate buffer from 10:90 to 50:50 over 15 min.

Flow rate: 1.5

Injection volume: 20

Detector: UV 190-370

CHROMATOGRAM

Retention time: 10.5

OTHER SUBSTANCES

Extracted: morphine, ephedrine, phenylpropanolamine, diphenhydramine, nortriptyline, lidocaine, benzoylecgonine, norpropoxyphene, nordiazepam

Also analyzed: amitriptyline, amphetamine, meperidine, codeine, (different gradient)

REFERENCE

Li,S.; Gemperline,P.J.; Briley,K.; Kazmierczak,S. Identification and quantitation of drugs of abuse in urine using the generalized rank annihilation method of curve resolution, *J.Chromatogr.B*, **1994**, *655*, 213–223.

SAMPLE

Matrix: vitreous humor

Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH, 1 mL water, and 500 μL 10 mM pH 3.4 phosphoric acid. 500 μL Vitreous humor + 250 μL 10 mM phosphoric acid, add to SPE cartridge, wash with 1 mL 10 mM phosphoric acid, wash with 500 μL 100 mM acetic acid, wash with 500 μL MeOH, wash with 500 μL 300 mM ammonium hydroxide, elute with 2 mL MeOH. Evaporate eluate to dryness at 70° under nitrogen, reconstitute in 500 μL 20 μg/mL tetracaine hydrochloride in MeOH, inject a 25 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 Lichrospher 100 RP-18

Column: 125 × 4 Lichrospher 100 RP-18

Mobile phase: MeOH:buffer 75:25 (Buffer was 320 mL 20 mM K_2HPO_4 + 680 mL 20 mM KH_2PO_4, pH 7.)

Flow rate: 0.6

Injection volume: 25

Detector: UV 235

CHROMATOGRAM

Retention time: 6.40

Internal standard: tetracaine (9.40)

Limit of detection: 500 ng/mL

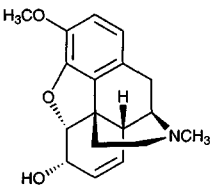
OTHER SUBSTANCES

Extracted: benzoylecgonine

KEY WORDS
SPE

REFERENCE
Fernández,P.; Rodríguez,P.; Bermejo,A.M.; López-Rivadulla,M.; Cruz,A. Simultaneous determination of cocaine and benzoylecgonine in vitreous humor by HPLC, *J.Liq.Chromatogr.*, **1994**, *17*, 883–890.

Codeine



Molecular formula: $C_{18}H_{21}NO_3$

Molecular weight: 299.37

CAS Registry No.: 76-57-3, 6069-47-8 (monohydrate), 5913-71-3 (acetate), 125-25-7 (HBr), 1422-07-7 (HCl), 6020-73-1 (salicylate), 125-27-9 (methyl bromide), 52-28-8 (phosphate), 41444-62-6 (phosphate hemihydrate), 1420-53-7 (sulfate), 6854-40-6 (sulfate trihydrate)

Merck Index: 2525

Lednicer No.: 1 287; 2 317

SAMPLE

Matrix: blood

Sample preparation: Condition a Certify SPE cartridge (Varian) with 2 mL MeOH and 2 mL 100 mM pH 8.0 phosphate buffer. Vortex 500 μL plasma and 1 mL 100 mM pH 8.0 phosphate buffer for 10 min and add the sample to the SPE cartridge using a gentle vacuum. Wash with 5 mL 100 mM pH 8.0 phosphate buffer, 1 mL water, 2 mL 100 mM pH 4.0 acetate buffer, and 2 mL MeOH. Dry cartridge under vacuum, wash with 200 μL butyl chloride:isopropanol 80:20, dry under vacuum. Elute with 1.2 mL butyl chloride: isopropanol 80:20, evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase. Inject a 150 μL aliquot.

HPLC VARIABLES

Guard column: GuardPak C18 μBondapak

Column: 100 × 8 NovaPak C18

Mobile phase: MeCN:MeOH:13.3m M pH 7.5 phosphate buffer 2:23:75 containing 40 mg/L cetyltrimethylammonium bromide

Flow rate: 1

Injection volume: 150

Detector: E, Waters Model 460, working electrode 1.10-1.25 V, reference electrode KCl

CHROMATOGRAM

Retention time: 14.7

Internal standard: codeine

OTHER SUBSTANCES

Extracted: oxycodone

KEY WORDS

plasma; SPE; codeine is IS

REFERENCE

Wright,A.W.E.; Lawrence,J.A.; Iu,M.; Cramond,T.; Smith,M.T. Solid-phase extraction method with high-performance liquid chromatography and electrochemical detection for the quantitative analysis of oxycodone in human plasma, *J.Chromatogr.B*, **1998**, *712*, 169–175.

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL 100 mg ethyl SPE cartridge (J.T.Baker) with 2 mL MeOH, 1 mL water, and 2 mL 1 mM pH 9.3 ammonium hydrogen carbonate buffer. Mix 1 mL serum with 200 μL 1 μg/mL IS in water. Add to the SPE cartridge, wash with 1 mL 1 mM pH 9.3 ammonium hydrogen carbonate buffer, elute with 1 mL MeOH. Evaporate the eluate to dryness, reconstitute the residue in 100 μL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm Supelcosil LC-Si (Supelco)
Mobile phase: MeCN:MeOH:water:formic acid 5.2:59.8:34.65:0.35
Flow rate: 0.23
Injection volume: 5
Detector: MS, API I MS single quadrupole, ionspray, capillary tip 5000 V, interface plate 650 V, source 60°, positive ion mode, SIM, orifice 70 V, m/z 300

CHROMATOGRAM
Retention time: 22.09
Internal standard: nalorphine (15.40)
Limit of quantitation: 4 ng/mL

OTHER SUBSTANCES
Extracted: - diamorphine, morphine

KEY WORDS
serum; pharmacokinetics; SPE; mouse

REFERENCE
Zuccaro,P.; Ricciarello,R.; Pichini,S.; Pacifici,R.; Altieri,I.; Pellegrini,M.; D'Ascenzo,G. Simultaneous determination of heroin, 6-monoacetylmorphine, morphine, and its glucuronides by liquid chromatography-atmospheric pressure ionspray-mass spectrometry, *J.Anal.Toxicol.*, **1997**, *21*, 268–277.

SAMPLE
Matrix: blood, CSF, urine, vitreous humor
Sample preparation: Condition a 200 mg Bond Elut SPE cartridge with 1 mL MeOH, 1 mL water, and 2 mL buffer. Centrifuge 1.5 mL serum, CSF, urine, or vitreous humor at 14000 g for 5 min, vortex 1 mL supernatant with 2 mL buffer and 100 ng internal standard. Centrifuge at 5000 g for 10 min, slowly add 2 mL supernatant to the SPE cartidge, wash with 2 mL buffer, dry under vacuum for 5 min. Elute with 500 μL MeOH:500 mM acetic acid 90:10 under gravity. Dry the eluate under a stream of nitrogen, reconstitute in 100 μL mobile phase, centrifuge at 14000 g for 4 min, inject a 10-20 μL aliquot. (Prepare buffer by adjusting pH of 900 mL 960 mg/L ammonium carbonate to 9.3 with concentrated ammonium hydroxide and 1 M ammonium hydroxide, make up to 1 L.)

HPLC VARIABLES
Column: 125 × 3 4 μm Superspher RP 18
Mobile phase: MeCN:50 mM pH 3 ammonium formate buffer 5:95
Flow rate: 0.6 for 4 min, to 1.1 over 3 min, maintain at 1.1 for 10 min
Injection volume: 10-20
Detector: MS, Finnigan MAT SSQ 7000 single quadrupole, 100-500u, 10 V, positive ion, sheath gas nitrogen pressure 70 p.s.i., auxiliary gas nitrogen 20 mL/min; heated vaporizer temperature 400°, heated capillary temperature 170°, corona current, 5 μ A, m/z 300

CHROMATOGRAM
Retention time: 9.5
Internal standard: codeine-d6
Limit of detection: 2.5 ng/mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 6-monoacetylmorphine, morphine

KEY WORDS
serum; SPE

REFERENCE

Bogusz,M.J.; Maier,R.-D.; Erkens,M.; Driessen,S. Determination of morphine and its 3- and 6-glucuronides, codeine, codeine glucuronide and 6-monoacetylmorphine in body fluids by liquid chromatography atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.B*, **1997**, *703*, 115–127.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 212.2

CHROMATOGRAM

Retention time: 4.975

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 μBondapak C18

Mobile phase: MeCN:water adjusted to pH 3 with phosphoric acid 70:30

Flow rate: 1

Injection volume: 10

Detector: UV 210

CHROMATOGRAM

Internal standard: codeine phosphate

OTHER SUBSTANCES

Simultaneous: fentanyl

KEY WORDS
codeine is IS

REFERENCE
Vanbever,R.; Le Boulengé,E.; Préat,V. Transdermal delivery of fentanyl by electroporation. I. Influence of electrical factors, *Pharm.Res.*, **1996**, *13*, 559–565.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Adsorbosphere C18 (Alltech)
Mobile phase: MeCN:water 25:75 containing 15 μL triethylamine per 100 mL
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 9.7

OTHER SUBSTANCES
Also analyzed: diamorphine, fentanyl, meperidine, morphine

REFERENCE
Lichtman,A.H.; Meng,Y.; Martin,B.R. Inhalation exposure to volatilized opioids produces antinociception in mice, *J.Pharmacol.Exp.Ther.*, **1996**, *279*, 69–76.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.15

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: urine
Sample preparation: Dilute 200 μL rat urine with 1.8 mL water. 2 mL Human urine or diluted rat urine + 500 μL 25% ammonia, extract twice with 3 mL portions of cyclohexane: ethyl acetate 50:50 for 10 min. Centrifuge at 4000 rpm for 20 min, remove the organic layer and evaporate it under a stream of nitrogen. Reconstitute the residue in MeOH:50 mM pH 4.5 phosphate buffer 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4 RP Select B RP8 (Merck)
Column: 250 × 4 5 μm RP Select B RP8 (Merck)
Mobile phase: Gradient. A was MeOH. B was THF. C was 50 mM pH 4.5 phosphate buffer. A:B:C from 27:3:70 to 22:3:75 over 18.9 min, to 48:4:48 over 10 min.
Flow rate: 0.5 for 18.9 min then 0.45
Injection volume: 20
Detector: UV 259

KEY WORDS
human; rat; codeine is IS

REFERENCE
Prien,D.; Rehn,D.; Blaschke,G. Enantioselective biotransformation of the chiral antihistaminic drug dimethindene in humans and rats, *Arzneimittelforschung*, **1997**, *47*, 653–658.